REMARKS

I. STATUS OF THE CLAIMS

Claims 1-7 and 9-10 remain pending in the application. No claims have been allowed. No claims stand objected to. Claims 1-10 remain rejected. Claim 1 is amended herein. Claim 8 has been canceled. Claims 11-14 have been withdrawn from consideration. New Claims 15 and 16 have been added.

II. SUMMARY OF THE REJECTIONS

Claims 1-10 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking sufficient written description in the specification.

Claims 1-10 have been rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness.

Claims 1-4 and 7-9 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over U.S. Patent No. 5,856,117 to Uenoyama et al. ("Uenoyama") in view of U.S. Patent No. 5,384,247 to Berry et al. ("Berry").

Claims 5 and 6 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of GB Patent No. 2,204,398 A to May et al. ("May").

Claim 10 remains rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of U.S. Patent No. 6,130,055 to Nanbu et al. ("Nanbu").

Applicants respectfully traverse these rejections and request reconsideration.

III. SUMMARY OF THE INVENTION

The present invention is an assay for trypsin inhibitors in urine which involves contacting a urine test sample with a buffered assay medium comprising trypsin, a substrate for trypsin which will produce a detectable response when cleaved by trypsin and a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine test sample, and correlating the concentration of the trypsin inhibitor with the detectable response from the cleaving of the substrate.

Also included within the scope of the present invention is a dry assay device having trypsin, buffer, a trypsin substrate and a chelating agent in an absorbent carrier for detecting the presence and concentration of trypsin inhibitor in urine test samples.

IV. SUMMARY OF THE AMENDMENTS

Claim 1 is amended to recite a range for the trypsin concentration, trypsin substrate concentration, chelating agent concentration and pH buffering. Support for the amendments to Claim 1 is found in pending Claim 8 and in the specification at page 5, lines 4-12. Claim 8 is canceled.

New claim 15 recites that the buffer can be a phosphate group-containing buffer, a carboxyl group containing buffer or a Tris buffer. Support for these claims can be found in the specification, page 10, lines 14-19, and page 17, lines 14-16. New claim 16 recites that the buffer can be a phosphate group containing buffer or a carboxyl group-containing buffer.

The specification is amended to claim priority to U.S. Ser. No. 60/204,032.

No new matter is added.

V. ARGUMENTS IN SUPPORT OF PATENTABILITY

Claims 1-10 have been rejected under §112, first paragraph for an alleged lack of sufficient written description. The Examiner alleges that the application "does not disclose that the calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent." Office Action, pages 2-3. Applicants respectfully disagree.

Applicants have amended claim 1 to supplement the term "in sufficient quantity" by inserting a numerical concentration range for polycarboxylic chelating agent. Support for this amendment is provided in the specification at page 5, lines 4-12. Accordingly, this rejection is now moot and should be withdrawn.

Moreover, the specification does disclose that the calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium

present in the urine test sample with the polycarboxylic chelating agent. On page 2, lines 28-31, Summary of the Invention, the specification discloses "a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine test." The specification also teaches that the "assay of the present invention is based on the discovery that the interference with the urine trypsin assay caused by the presence of calcium ion in the urine can be factored out of the assay by the use of certain chelating agents." Specification, page 4, lines 21-30. In Example I, the specification discloses that the "maximum practical amount of calcium in urine was determined to be 80 mg/dL based on published data and double the amount of EGTA (0.47 g/L) to complex this amount of calcium was added to the assay system." Specification, page 7, lines 15-19. Later in Example I, after comparing samples containing polycarboxylic chelating agents with those lacking polycarboxylic chelating agents, it "was furter determined that the calcium either had to be overwhelmed or complexed to remove it from the assay system." Specification, page 10, lines 4-6. The assay system chosen was to complex the calcium, rather than precipitate the calcium. Specification, page 10, lines 7-10. Thus, the patent application does disclose conditions where the calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent.

The part of the specification cited by the Examiner (specification, page 10, lines 14-19, Example I following discussion of complexing calcium, see above) deals with precipitation of calcium. While the phosphate and carboxyl groups can advantageously reduce calcium concentration by precipitation, the specification also discloses other buffers that can be used in the assay system of the invention. Specification, page 17, lines 14-16.

in summary, the specification provides a sufficient written description to show that the Applicants had possession of the concept that the calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent. Applicants respectfully request that this rejection be withdrawn.

Claims 1-10 have been rejected under §112, second paragraph. The Examiner alleges that the term "in sufficient quantity" in claim 1 is vague and indefinite. Applicants have amended claim 1 to supplement the term "in sufficient quantity" by inserting a definite numerical concentration range for polycarboxylic chelating agent. The recited concentration of polycarboxylic chelating agent is a sufficient quantity to inhibit interference with the assay from calcium present in the urine as assay reagents. This rejection is now moot and should be withdrawn.

Claims 1-10 remain rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Uenoyama in view of Berry and further in view of May or Nanbu. Applicants respectfully traverse.

In response to the Applicants' arguments filed November 27, 2002, the Examiner alleges, regarding the quantity of calcium ions in the buffer medium of Claim 1, that "it is unclear what is considered to be in sufficient quantity and the claim as recited incorporates an amount of calcium still present". Office Action of February 12, 2003, Paper 14, bridging pages 6-7. The Examiner also alleges that 'the instant claims as recited do not recite that calcium is removed" and concludes "that the Uenoyama et al reference still reads on the instant claims." Office Action, page 7, second paragraph. Applicants respectfully disagree.

Applicants have amended the claims to recite, *inter alia*, that one component of the buffered assay medium is "a calcium free buffer wherein the pH of the buffered assay medium is buffered at a level of from 6.0 to 8.0, and wherein calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent." The term "calcium free buffer" is well known in the art to refer to buffers have low levels of free calcium ions. Calcium free buffers have long and commonly been used in physiological assays to prevent undesired calcium ion-dependent reactions. Examples of calcium free buffers are provided in the specification at pg. 10, lines 14-19:

> Phosphate and carboxyl groups are common as the charged ionizable groups of buffering agents and calcium salts of these groups are not very water soluble (calcium phosphate is relatively insoluble), so they tend to precipitate from solution.

Phosphate monobasic buffer adjusted to pH 7.8 with sodium hydroxide is described in the specification, pg. 24. A different kind of calcium free buffer, Tris, is described in the specification, pg. 17, lines 14-16.

Additional guidance as to what a "sufficient quantity" of calcium would be to interfere with the chelating agent is found in the specification. "The maximum practical amount of calcium in urine was determined to be about 80 mg/dL based on published data and double the amount of EGTA (0.47 g/L) needed to complex this amount of calcium was added to the assay system." Specification at page 7, lines 15-19.

Accordingly, the claims as amended are distinguished from the Uenoyama reference, in which the buffer solution contains "at least 0.15 µmol per 1 µg of protease". Uenoyama, col. 2, line 44. As an example, Uenoyama uses a TEA buffer containing dissolved calcium salt. Uenoyama, col. 7, lines 50-51. By contrast, the calcium free buffers in the claimed assay either remove calcium from the assay medium (*e.g.*, for buffers containing phosphate or carboxyl groups) or else contain a low level of free calcium ions that there is no interference with the binding of calcium present in the urine test sample to the polycarboxylic chelating agent.

The Examiner also alleges, in response to the Applicants' arguments filed November 27, 2002, that Berry teaches a method of assaying for calcium ions that uses a selective binding agent for the binding of interfering ions. Office Action, page 7, third paragraph. The Examiner cites Berry, in the Abstract and at col. 4, lines 53-55. Applicants respectfully disagree.

The principle of the Berry assay is different from the assay of the invention. Applicants note that the Abstract states that an "essential feature is a method to exclude interferences by ions by masking the interfering ions with a binding agent." Berry, Abstract. By contrast, the claimed invention does not "mask" calcium, as disclosed in the specification.

> The assay of the present invention is based on the discovery that the interference with the urine trypsin assay caused by the presence of calcium ion in the urine can be factored out of the assay by the use of certain chelating agents. This was unexpected because the chelating agents were not used to extract and remove calcium but only to complex the salt. It was to be expected that trypsin would still interact with the complexed salt in a detrimental fashion.

Specification, pg. 4, lines 21-30. Other than the act of binding calcium, the chelating agents perform different functions in the respective assays. Accordingly, one of skill in the art would not have combined Berry with Uenoyama to develop a calcium assay.

Moreover, Berry does not disclose the other advantages of the use of chelating agents that are disclosed in the specification. For example, the use of "EGTA reduces variation between urine samples having increasing amounts of calcium." Specification, page 9, lines 29-31, discussing the results presented in Tables A and B. Moreover, a "formula with EGTA is less prone to the effects of surfactant than without." Specification, page 16, lines 32-34, discussing the results presented in Tables C and D. While these considerations are not disclosed by Berry, they are important to the present invention as a whole.

In summary, the claimed invention, as amended, is not obvious in view of the cited references. Applicants respectfully request withdrawal of all the rejections under 35 U.S.C. § 103.

VI. CONCLUSION

These amendments and remarks remove all grounds for rejection. Applicants request allowance of the remaining pending claims.

Applicants' attorney invites the Examiner to telephone if he has any questions about the application or this submission.

Respectfully submitted,

By: _____

John Prince
Attorney for Applicants
Reg. No. 43,019

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Date

McDermott, Will & Emery
28 State Street
Boston, MA 02109-1775
(617) 535-4435
(617) 535-3800